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## **In vitro evaluation of the erosive potential of viscosity-modified soft acidic drinks on enamel**

Aykut-Yetkiner, Arzu ; Wiegand, Annette ; Ronay, Valerie ; Attin, Rengin ; Becker, Klaus ; Attin, Thomas

**Abstract:** **OBJECTIVE:** The objective of this in vitro study was to investigate the effect of viscosity-modified soft acidic drinks on enamel erosion. **MATERIALS AND METHODS:** A total of 108 bovine enamel samples ( $\approx 3$  mm) were embedded in acrylic resin and allocated into six groups ( $n = 18$ ). Soft acidic drinks (orange juice, Coca-Cola, Sprite) were used both in their regular forms and at a kinetic viscosity of  $5 \text{ mm}^2/\text{s}$ , which was adjusted by adding hydroxypropyl cellulose. All solutions were pumped over the enamel surface from a reservoir with a drop rate of  $3 \text{ ml/min}$ . Each specimen was eroded for 10 min at  $20^\circ\text{C}$ . Erosion of enamel surfaces was measured using profilometry. Data were analyzed using independent  $t$  tests and one-way ANOVAs ( $p < 0.05$ ). **RESULTS:** Enamel loss was significantly higher for the regular (Coca-Cola,  $5.60 \pm 1.04 \text{ mm}$ ; Sprite,  $5.49 \pm 0.94 \text{ mm}$ ; orange juice,  $1.35 \pm 0.4 \text{ mm}$ ) than for the viscosity-modified drinks (Coca-Cola,  $4.90 \pm 0.34 \text{ mm}$ ; Sprite,  $4.46 \pm 0.39 \text{ mm}$ ; orange juice,  $1.10 \pm 0.22 \text{ mm}$ ). **CONCLUSION:** For both regular and viscosity-modified forms, Coca-Cola and Sprite caused higher enamel loss than orange juice. Increasing the viscosity of acidic soft drinks to  $5 \text{ mm}^2/\text{s}$  reduced enamel erosion by 12.6-18.7 %. **CLINICAL RELEVANCE:** The erosive potential of soft acidic drinks is not only dependent on various chemical properties but also on the viscosity of the acidic solution and can be reduced by viscosity modification.

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**In vitro evaluation of the erosive potential of viscosity modified soft  
acidic drinks on enamel**

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## ABSTRACT

**Objective** The objective of this *in vitro* study was to investigate the effect of viscosity-modified soft acidic drinks on enamel erosion. **Materials and Methods** A total of 108 bovine enamel samples ( $\varnothing=3$  mm) were embedded in acrylic resin and allocated to 6 groups (n=18). Soft acidic drinks (orange juice, Coca-Cola, Sprite) were used both in their regular forms and at a kinetic viscosity of  $5 \text{ mm}^2/\text{s}$ , which was adjusted by adding hydroxypropyl cellulose (HPC). All solutions were pumped over the enamel surface from a reservoir with a drop rate of 3ml/min. Each specimen was eroded for 10 min at  $20^\circ\text{C}$ . Erosion of enamel surfaces was measured using profilometry. Data were analyzed using independent t-tests and one-way ANOVAs ( $p<0.05$ ). **Results** Enamel loss was significantly higher for the regular (Coca-Cola:  $5.60 \pm 1.04 \mu\text{m}$ , Sprite:  $5.49 \pm 0.94 \mu\text{m}$ , orange juice:  $1.35 \pm 0.4 \mu\text{m}$ ) than for the viscosity-modified drinks (Coca-Cola:  $4.90 \pm 0.34 \mu\text{m}$ , Sprite:  $4.46 \pm 0.39 \mu\text{m}$ , orange juice:  $1.10 \pm 0.22 \mu\text{m}$ ). **Conclusion** For both regular and viscosity-modified forms, Coca-Cola and Sprite caused higher enamel loss than orange juice. Increasing the viscosity of acidic soft drinks to  $5 \text{ mm}^2/\text{s}$  reduced enamel erosion by 12.58-18.71%. **Clinical Relevance** The erosive potential of soft acidic drinks is not only dependent on various chemical properties, but also on the viscosity of the acidic solution and can be reduced by viscosity-modification.

**Keywords:** enamel erosion, viscosity, cellulose, soft acidic drinks

## INTRODUCTION

The frequent consumption of acidic drinks and foods cause extrinsic erosive lesions, which have been shown to be increasingly present in children [1] and young adults [2] with a high intake of acidic soft drinks.

As the erosive potential of acidic beverages is determined by various chemical factors including pH, buffer capacity, chelating abilities and mineral content, it seems evident that specific modifications of these chemical factors might reduce their erosive potential.

A common approach is to increase the degree of saturation of erosive drinks by adding calcium, phosphate and/or fluoride ions, or by supplementation with hydroxyapatite as a source of calcium and phosphate [3–5]. Thereby, the addition of 1 mmol/l Ca or a combination of 0.5 mmol/l Ca, 0.5 mmol/l P and 0.0031 mmol/l F resulted in a 38 – 64% reduction of erosive enamel loss [3].

It has been also shown that the addition of food proteins (casein and ovalbumin) reduced enamel erosion [6], probably due to adsorption of protein to the hydroxyapatite surface [7]. The proteins might form a semi-permeable barrier, hindering transport of protons towards the hydroxyapatite crystal surface and/or calcium and phosphate ions away from the surface [8].

Another approach to reduce the dentin erosion is the addition of green tea extract containing epigallocatechin-3-gallate (ECGG), a polyphenol that inhibits matrix metalloproteinases (MMP-2 and MMP-9) [9]. The inhibition of MMPs is known to reduce the enzymatic degradation of the organic matrix of demineralized dentin [10, 11].

More recently, the addition of specific polymers like xanthan gum, carboxymethyl cellulose, pectin, alginate or gum arabic polymers was tested

to reduce the erosive potential of acids [12,13] or acidic drinks [14,15]. The efficacy of the polymers was related to the formation of a protective layer at the enamel surface, probably by the interaction between negatively charged carboxyl groups of polymers and calcium ions of the enamel surface, or by a chelating complex of the polymers and calcium [13]. However, in a recent in situ study, calcium lactate pentahydrate and linear sodium polyphosphate-(LPP) failed to reduce enamel erosion significantly. It was assumed that under clinical conditions the adsorption of LPP to the enamel surface was suppressed by the presence of some salivary proteins [16].

In contrast, the modification of physical or physico-chemical aspects of erosive solutions has been hardly investigated. It is known that dental erosion is affected by the temperature and the acid flow rate of the acidic solutions [17]. In a previous study it was shown that dental erosion is also dependent on the viscosity of acidic solutions [18].

Thereby, even small changes in acid viscosity (increase from viscosity 1.5 to 6 mm<sup>2</sup>/s) resulted in a reduction of erosive enamel loss of about 10 -15%. As pure acidic solutions were used in the previous study, it is not known, in how far these observations might be transferred to commercial soft drinks, which shows up with multiple ingredients.

Therefore, the aim of this in vitro study was to investigate the effect of the modification of the native viscosity of soft acidic drinks to 5 mm<sup>2</sup>/s on enamel erosion. The null hypothesis was that the erosivity of soft drinks is not affected by the increase of their viscosity.

## MATERIALS METHODS

### **Specimen preparation**

Specimens were obtained from intact bovine incisors. The crowns were separated from roots and stored in 0.5% thymol solution for a maximum of 6 months at 5°C until used. One hundred and eight bovine enamel specimens were prepared from the crowns using a water-cooled trephine bur and embedded in acrylic resin blocks (6 mm in diameter, Paladur, Heraeus Kulzer, Germany). All resin blocks had a notch, which fits in the metal jig of the profilometer preventing the rotation of the specimen and allowing exact repositioning. The enamel surfaces were then ground with water-cooled carborundum discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Struers, Erkrath, Germany). The specimens were randomly allocated to 6 groups of each  $n = 18$  specimens. The sample size of  $n = 18$  was determined based on a pilot experiment. This power calculation was based on a difference in means of 0.9  $\mu\text{m}$  detected at 95% power, considering that the standard deviation of one group (regular drink) is 0.94 and of the other group (viscosity-modified drink) is 0.28.

### **Modification of soft drinks**

The beverages evaluated were Coca-Cola (Coca Cola, Coca-Cola HBC Schweiz AG, Brüttisellen, Switzerland), Sprite (Sprite, Coca-Cola HBC Schweiz AG, Brüttisellen, Switzerland) and orange juice (Hohes C, Eckes-Granini, Henniez, Switzerland), which were tested plain (control) and after supplementation of hydroxypropyl cellulose (HPC, Grade LM, HPC, Nippon Soda Company, Japan) to increase the viscosity to 5  $\text{mm}^2/\text{s}$ . Therefore, the

beverages were decarbonated by stirring, and the HPC was then added by stirring at a room temperature of 20 for at least 60 min. By adding 15 g of HPC to a 1000 ml of Coca-Cola and Sprite, a viscosity of  $>6.5 \text{ mm}^2/\text{s}$  was obtained and by adding 13 g of HPC to a 1000 ml of orange juice a viscosity of  $>9.5 \text{ mm}^2/\text{s}$  was obtained. These new beverages were then diluted with the respective acid until the targeted viscosity was reached.

The pH-values and titratable acidities (TA) to pH 5.5 of the respective beverages before and after the addition of HPC were checked with a titrator (Metrohm, Herisau, Switzerland). The viscosities of the beverages with and without addition of HPC were measured ten times with a viscometer (Becker Research Equipment, Göttingen, Germany), which is described in detail in a previous study [19] and the average value was used. The initial and modified viscosities, pH levels and TAs were shown in Table 1.

As the viscosity is highly dependent on the temperature [20], measurements were taken at controlled room temperature of 20°C. The temperature of the beverages was adjusted to 20°C, also.

### **Experimental procedure**

From each specimens, five baseline profiles were recorded with a profilometer (Perthometer S2; Mahr, Göttingen, Germany) with a distance of 50  $\mu\text{m}$  between each profile. To allow repositioning of the samples at baseline and final profilometric measurements, the profilometer was equipped with a custom-made jig.

The respective beverages were pumped from a reservoir with a flow rate of 3 ml/min into an inclined channel (depth: 0.68 mm, width: 2 mm, length: 80

mm), which was made from poly vinyl chloride (Figure 1). The specimens were eroded for 10 min at a flow rate of 3ml/min, this means that a total amount of 30 ml flowed on each sample. The erosion treatment was conducted at a constant room temperature of 20°C. Afterwards, the specimens were rinsed with distilled water and the final profiles were taken. Enamel loss was calculated by a custom-made software (4D Client, custom designed software; University Zurich, Zurich, Switzerland), allowing for an exact matching of the baseline profiles with the respective final erosion profiles. The reproducibility of the repeated measurements was checked previously, and the vertical difference of each profile with regard to the first profile was found with a range of  $0 \pm 0.031 \mu\text{m}$  [21].

### **Data analysis**

Descriptive statistics (mean, SD) were computed, and normality was checked with Kolmogorov-Smirnov and Shapiro-Wilk's tests. As normal distribution was not found in all groups, the comparison between original and viscosity modified drinks was done by independent t-test. Erosive beverages at the same viscosity were compared with one-way ANOVAs.

The level of significance was set at  $\alpha = 0.05$ .

## **RESULTS**

Mean enamel loss caused by the original and by the modified beverages is presented in Figure 2. Overall, the regular drinks caused significantly higher enamel loss than the viscosity-modified drinks (Coca-Cola,  $p = 0.000$ ; Sprite,



$p = 0.002$ , orange juice,  $p = 0.013$ ). Thereby, erosive enamel loss was decreased by 12.6% for Coca-Cola and 18.7% for Sprite.

Generally significantly, highest loss was observed for Coca-Cola and Sprite compared to orange juice.

## DISCUSSION

Many studies tried to modify the erosivity of acidic drinks by taking only the chemical aspects into account. To the authors' best knowledge, this is the first study reporting that the erosive potential of soft acidic drinks is not only dependent on various chemical properties, but also on the viscosity of the acidic solution. The null hypothesis cannot be accepted.

The beverages tested in this study set-up were chosen due to their acidic nature, well-documented erosive potential [22,23] and worldwide consumption. The original viscosities of the tested beverages were regulated by adding hydroxypropyl cellulose (HPC), which is a derivative of polysaccharide cellulose and widely used in food, drug and cosmetics areas to obtain targeted viscosity of products [24]. The set up of the present study allowed the free flow of soft drinks over the surface of enamel specimens, so that the flow rate of the drinks were not defined by any pump system, rather it was determined by the viscosity of the liquid itself. The viscosity of the fluids [20] and also the erosive potential of acids [25] is directly related to the temperature of fluids, thus the experiments were undertaken at a standardized temperature of 20°C, and also the temperature of the tested solutions was adjusted to 20°C.

In all experimental groups the viscosity-modified drinks caused lower erosion than the regular drinks. It is assumed that the relative stickiness of the soft drinks with higher viscosity decreased the ion exchange and clearance of dissolution products. At high viscosity and, thus, reduced flow velocity, this static layer might be thicker and less undersaturated, so that enamel erosion decreased. However, it is not known so far, if there is also any interaction between the HPC polymer and the enamel surface, which might influence the erosive potential of the HPC-modified drinks. The addition of HPC to the original drinks caused only a very small change in the pH levels and titratable acidities, which were unlikely to affect the erosive potential significantly.

HPC belongs to the group of water-soluble polymers [26]. Water soluble polymers like xanthan gum, pectin, carboxymethyl cellulose were assumed to reduce enamel erosion [13, 14, 15, 27] by forming a gel-like polymer layer on enamel surfaces [13, 27]. In the present study, the used polymer HPC was known to be non-ionic, so it is unlikely to bind to enamel, which was reported in a previous study [18].

Previously, it has been reported that the decrease in the velocity of the erosive liquid flow reduces the amount of tissue loss due to erosion [17,28]. Possibly, this might be one of the contributing factors reducing erosion in the present study, since the velocity of the fluid flow on the enamel surface was also reduced due to the increased viscosity. However, the velocity of the liquids under the defined conditions was not determined in the present set-up. Besides the reduction of the erosive potential, the modification of drinks might lead to an alteration in the taste [3, 29, 30]. As the taste is an important criterion for a successful dietary modification [30] the authors of the present

study were asked for estimation of the taste of the viscosity-modified beverages compared to the original decarbonized ones. Most of the authors (87%) did not report any taste change for the orange juice. However, the viscosity-modified soft drinks were found to be different in taste (Coca-Cola: 66.7%, Sprite: 67.7%) or to have a more viscous feeling on the tongue (Coca-Cola: 17.7%, Sprite: 17.7%).

The change of the taste by adding thickeners to a beverage has been addressed previously [31]. In a study by Matta *et al.*, [31] starch based and gum based thickeners were added into coffee, milk, apple juice and orange juice to achieve nectar-like and honey-like consistencies. It was shown that all thickeners suppressed the main flavors of the base beverages and changed the taste (bitter, sour, metallic or astringent) in some beverages. Perception of drinks does not depend solely on taste; texture and organoleptic factors are important factors as well. By modifying the viscosity and (de)carbonization of a soft acidic drink, the taste itself might not differ to the consumer but the overall perception might get affected [32].

In conclusion, the present study showed that viscosity-modification of soft acidic drinks to 5 mm<sup>2</sup>/s reduced enamel erosion by 12.6 to 18.7%. However, this positive effect was partly counteracted by a change of taste of the viscosity-modified soft drinks.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Table 1. The viscosities [mm<sup>2</sup>/s], pH levels and titratable acidities (TA) of the original and modified soft drinks.

Drink	original			modified		
	visc	pH	TA	visc	pH	TA
Coca-Cola	1.15 ± 0.06	2.44	5.82	5.01 ± 0.23	2.47	5.86
Sprite	1.04 ± 0.04	2.65	24.13	5.08 ± 0.32	2.67	24.33
Orange juice	1.70 ± 0.16	3.72	81.00	5.02 ± 0.20	3.74	83.00

### **Figure Captions**

Figure 1. Flow incline ( $45^\circ$ ) allowing for rinsing specimens in parallel. The beverages were pumped from a reservoir with a flow rate of 3 ml/min.

Figure 2. Enamel loss ( $\mu\text{m}$ , mean  $\pm$  standard deviation) caused by original and high viscosity ( $\text{mm}^2/\text{s}$ ) soft drinks

Within the regular drinks and within the viscosity-modified drinks, significant differences between the groups were marked by different letters.

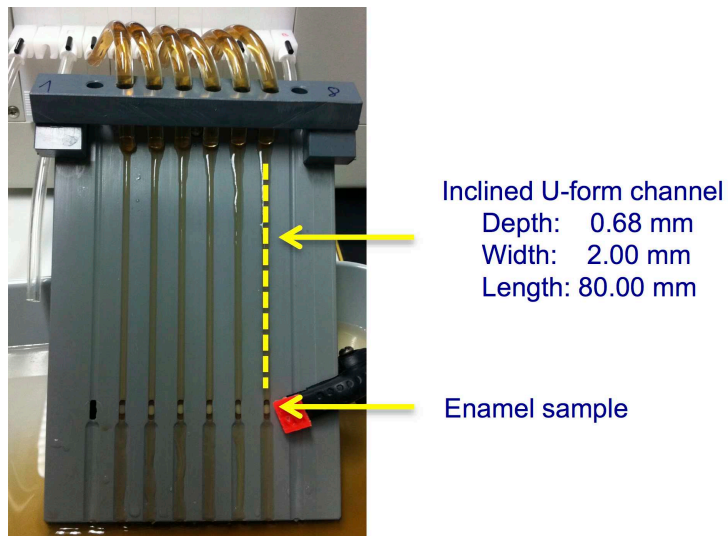


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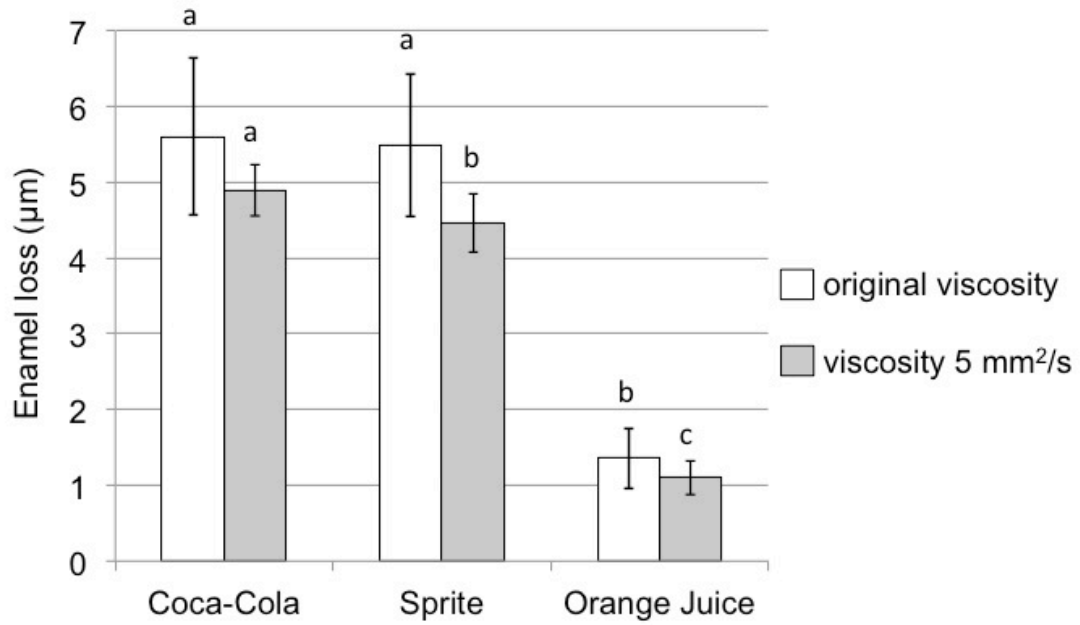


Figure 2. Enamel loss (µm, mean ± standard deviation) caused by original and high viscosity (mm<sup>2</sup>/s) soft drinks

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